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PRECIPITIN-PRODUCTION IN ALLERGIC RABBITS *

LUDVIG HEKTOEN

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In the course of efforts to develop in rabbits by the intraperitoneal injection of large quantities of horse blood or horse serum precipitins which would detect minute quantities of horse protein, it was observed that the introduction of horse blood or serum in rabbits treated a considerable time previously with sheep blood resulted in the production not only of precipitin for horse protein, but also for sheep protein, as well as for proteins of several other species. The wide range of the precipitating action of the serum rendered it quite unsuitable for the detection of horse protein in the presence of proteins from various other sources. Unless carefully and thoroughly studied, the use, for precipitin tests, of serum obtained under these and similar conditions might lead to highly misleading results.

These statements are based on a number of observations, some of which may be given in detail by way of illustration:

1.—Two rabbits each received 30 c.c. of sheep blood intraperitoneally on Sept. 23, 1915. Both developed a goodly quantity of precipitin for sheep blood as shown in Chart 1. Here as in the other charts the titer gives the highest dilution in salt solution of laked blood—sheep, horse, etc.—with restored salt content, in contact with which 0.1 c.c. of rabbit serum gave a definite precipitate after 1 hour at room temperature. The curves are quite typical: The highest concentration was reached about the 11th day after the injection and by about the 90th day—December 23—the serum appeared to have lost its precipitating effect. Tests with other protein solutions than sheep blood were not made during this time. On Feb. 8, 1916, 25 c.c. of horse blood were injected intraperitoneally and there developed, as shown also in Chart 1, precipitin in high concentration for horse blood, the dilutions of the horse blood and the tests being made in the same way as in the case of the sheep blood. Tests were now made in the same manner with the blood of other species and the results of 1 test are given on the chart, the figures representing, as stated, the highest dilutions of blood in which precipitates appeared. Several other tests were made with practically the same outcome.

We see that in these 2 rabbits the injection of horse blood some 5 months after the sheep blood, and several weeks after the precipitin for sheep blood had disappeared from the serum, resulted in the development of precipitins not only for horse blood, but also in about

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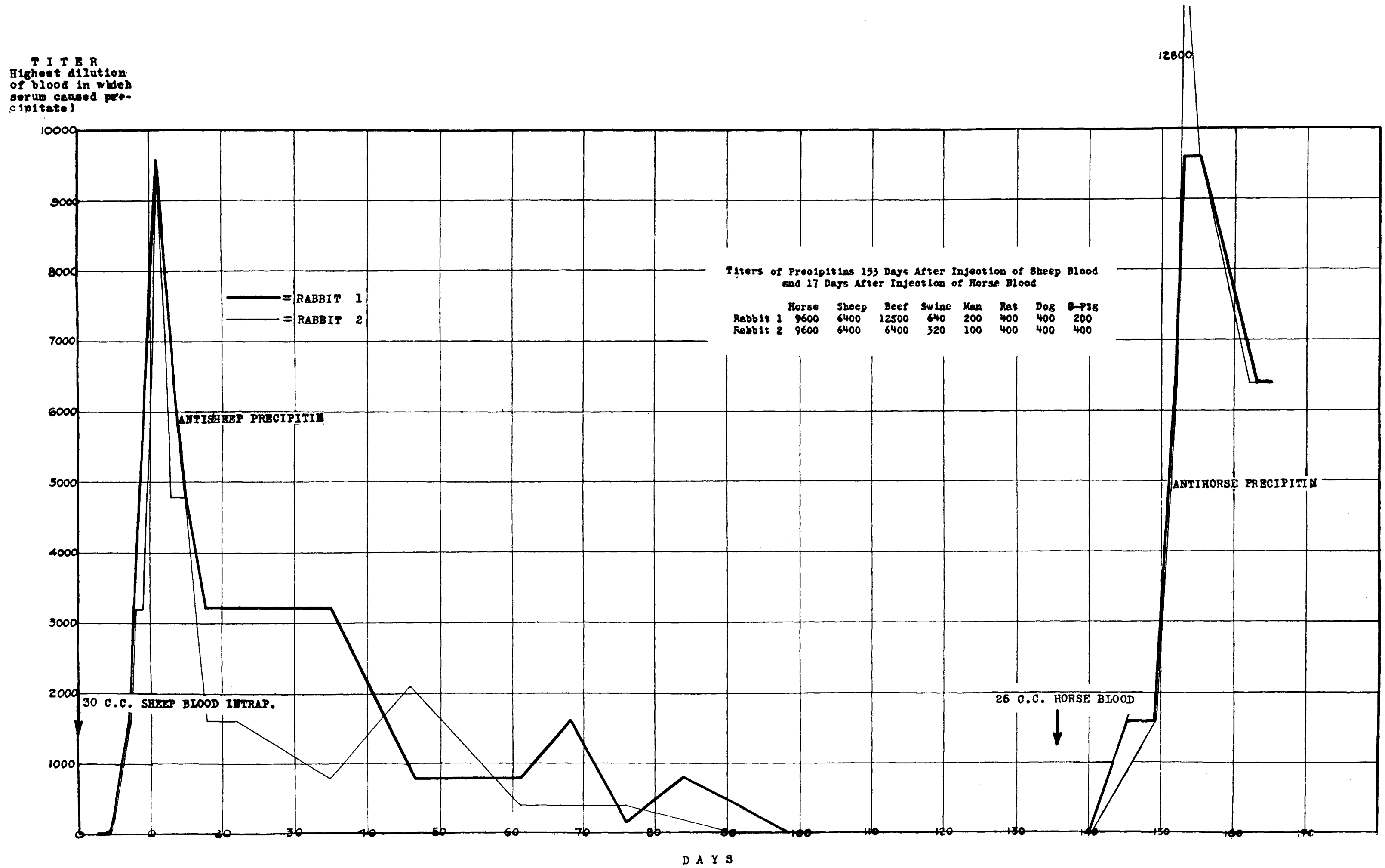
* Received for publication April 13, 1917.



Titers of Precipitins 153 Days After Injection of Sheep Blood and 17 Days After Injection of Horse Blood

	Horse	Sheep	Beef	Swine	Man	Rat	Dog	G-Pig
Rabbit 1	9600	6400	12500	640	200	400	400	200
Rabbit 2	9600	6400	6400	320	100	400	400	400

Chart 1.—Precipitins in 2 rabbits injected with sheep blood, and 140 days later with horse blood.

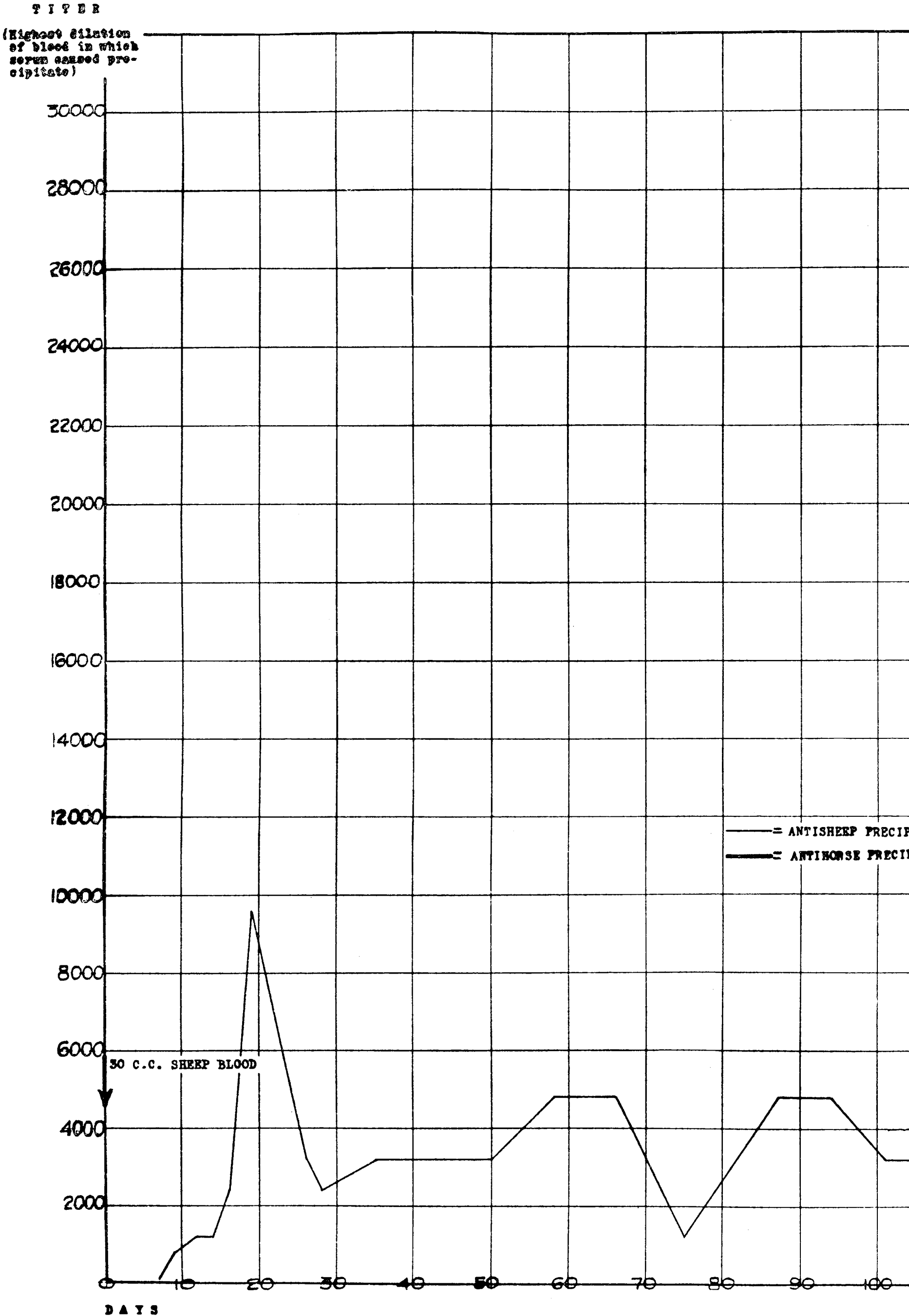
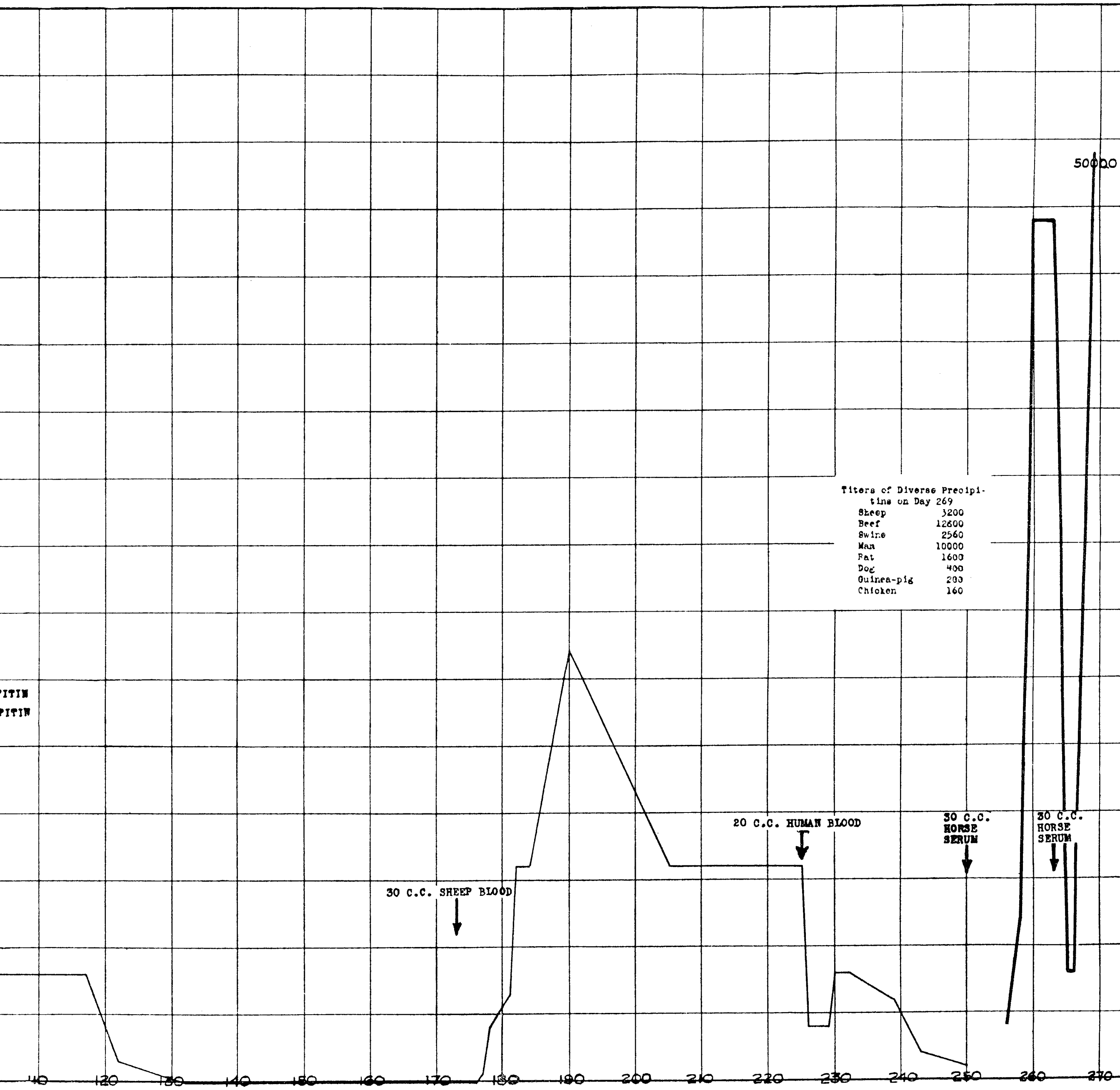
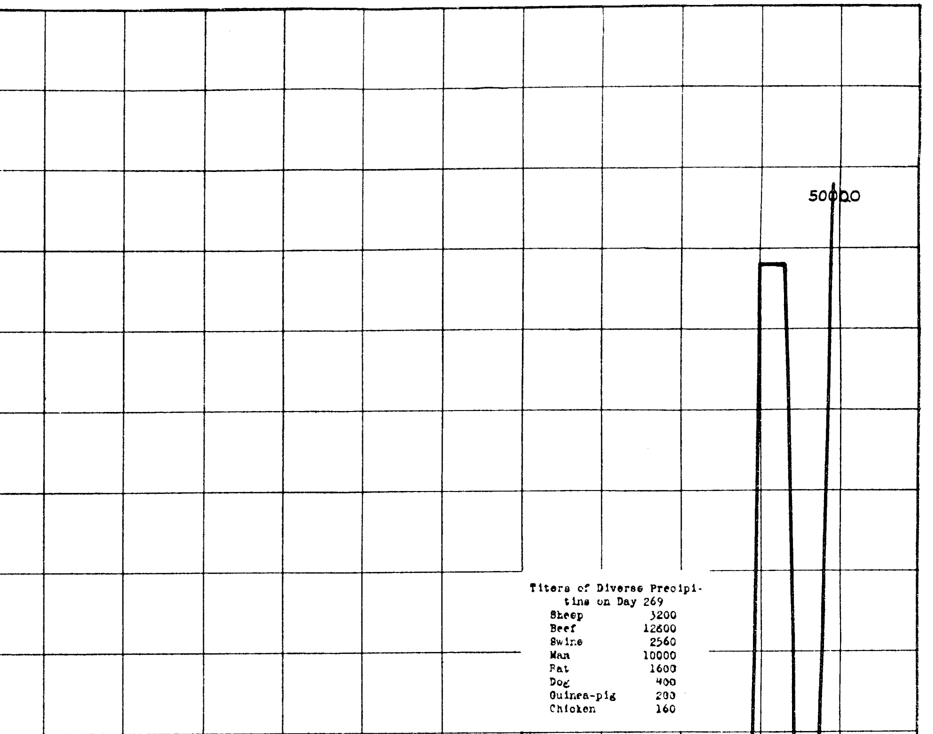


Chart. 2. Precipit



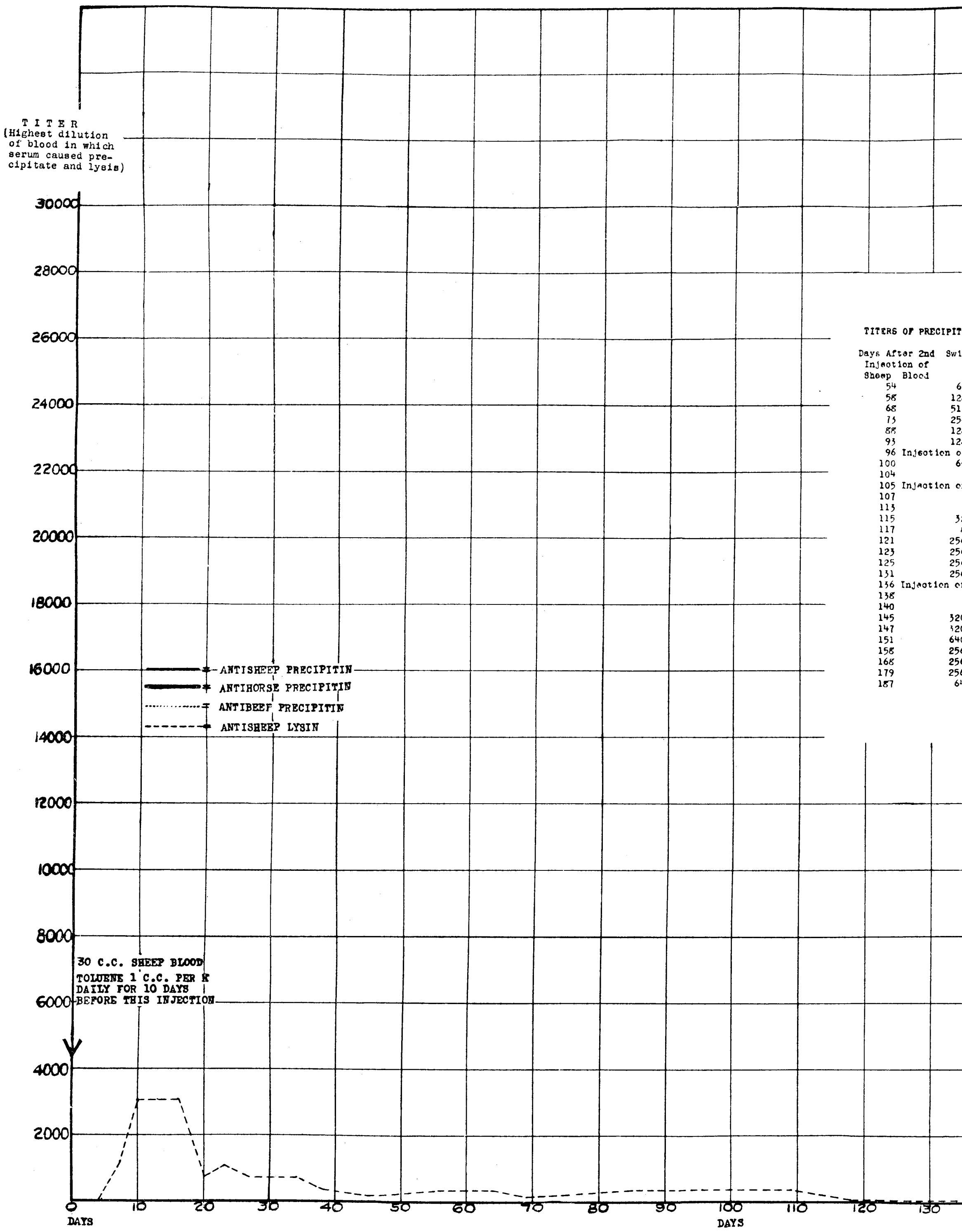
tins in rabbit injected with sheep blood, human blood, and horse serum.



Titers of Diverse Precipitins on Day 269

Sheep	3200
Beef	12800
Swine	2560
Man	10000
Rat	1600
Dog	400
Guinea-pig	200
Chicken	160

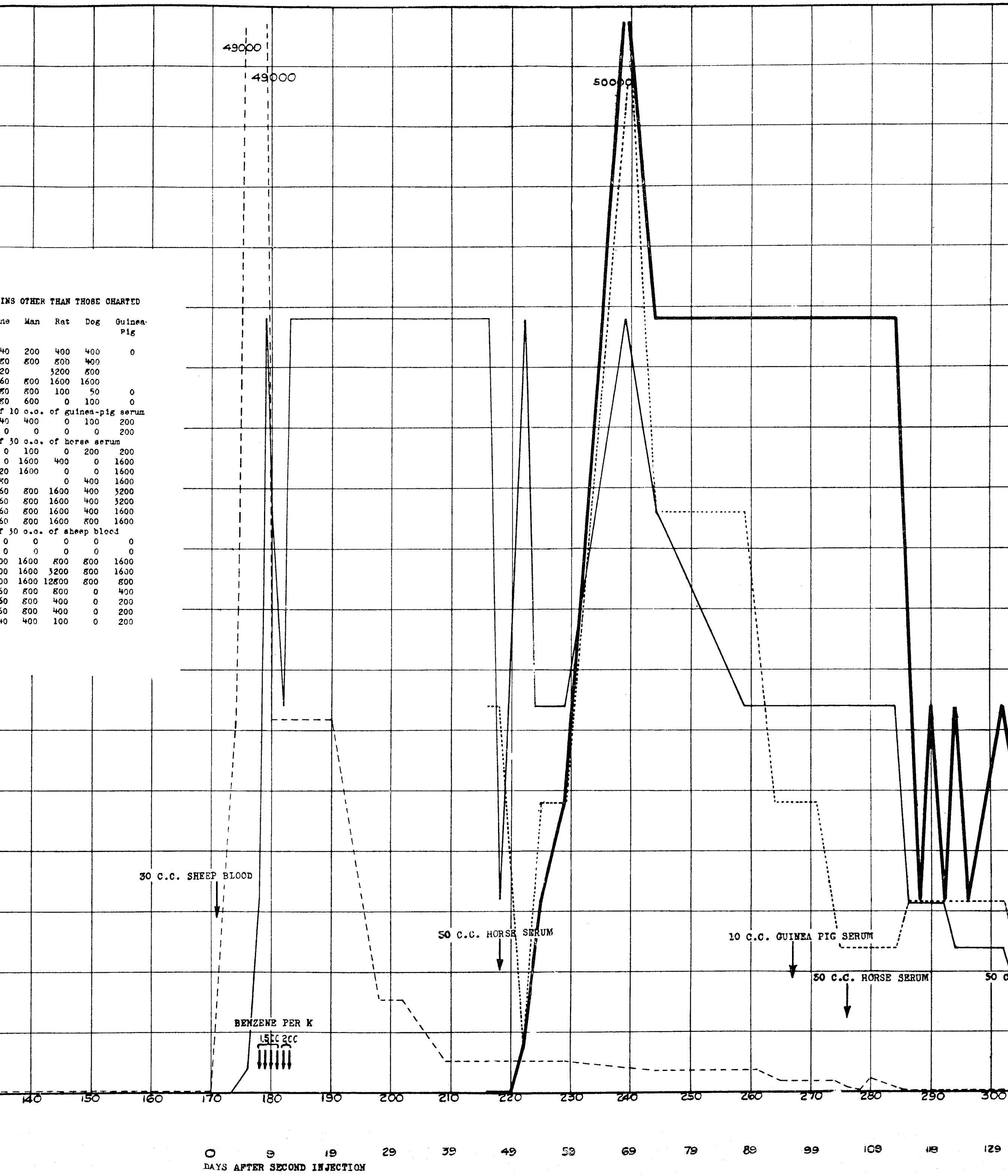
and horse serum.



TITERS OF PRECIPIT

Days After 2nd Injection of Sheep Blood	Swi
54	6
58	12
68	51
73	25
88	12
93	12
96	Injection o
100	6
104	
105	Injection o
107	
113	
115	3
117	
121	25
123	25
125	25
131	25
136	Injection o
138	
140	
145	32
147	32
151	64
158	25
168	25
179	25
187	6

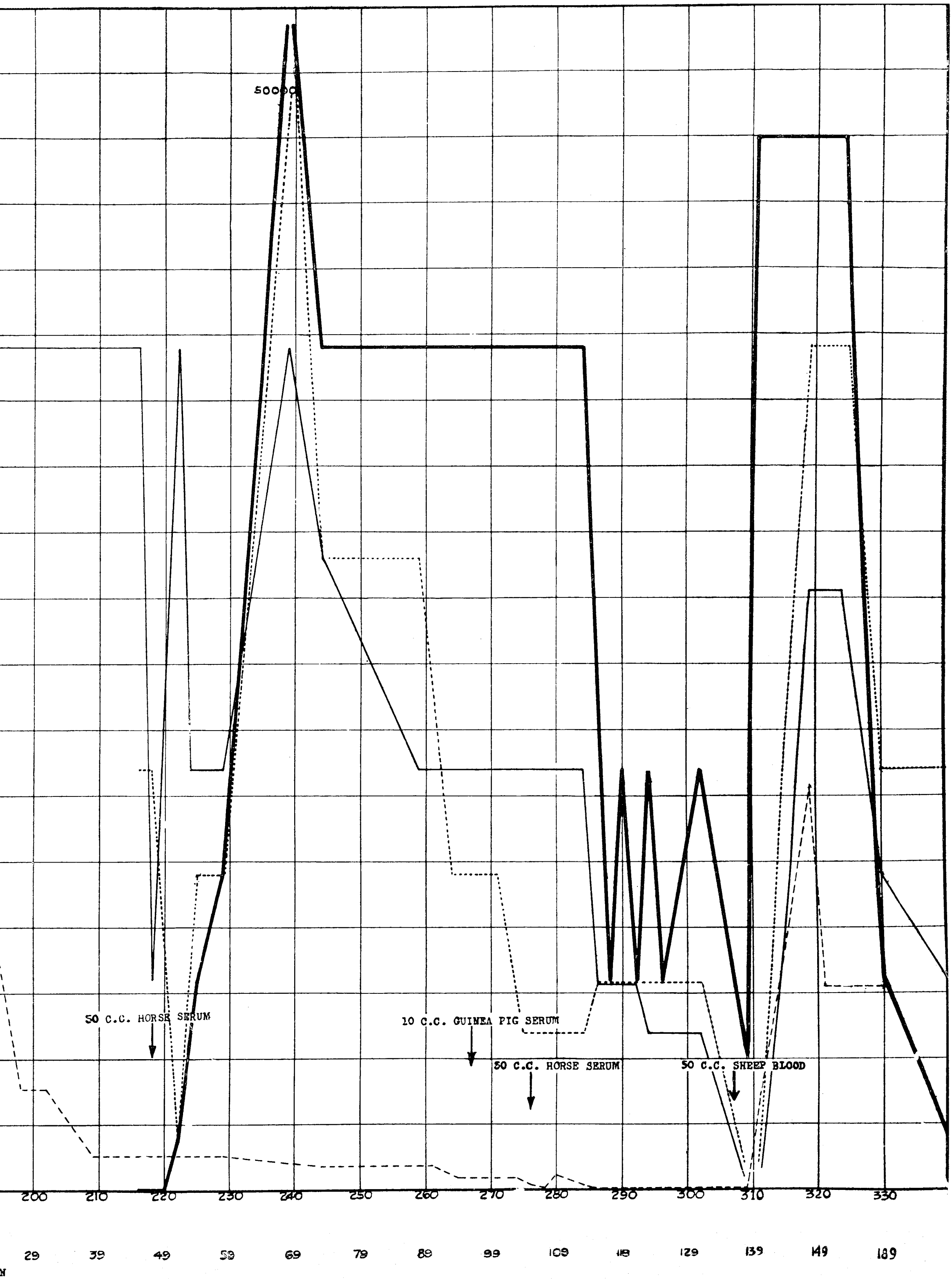
Chart. 3. Lysin



INS OTHER THAN THOSE CHARTED

ne	Man	Rat	Dog	Guinea-Pig
40	200	400	400	0
80	800	800	400	
20		3200	800	
60	800	1600	1600	
80	800	100	50	0
80	600	0	100	0
f 10 c.c. of guinea-pig serum				
40	400	0	100	200
0	0	0	0	200
f 30 c.c. of horse serum				
0	100	0	200	200
0	1600	400	0	1600
20	1600	0	0	1600
80		0	400	1600
60	800	1600	400	3200
60	800	1600	400	3200
60	800	1600	400	1600
60	800	1600	800	1600
f 30 c.c. of sheep blood				
0	0	0	0	0
0	0	0	0	0
00	1600	800	800	1600
00	1600	3200	800	1600
00	1600	12800	800	800
60	800	800	0	400
60	800	400	0	200
60	800	400	0	200
40	400	100	0	200

and precipitins in rabbit injected with sheep blood, horse serum, etc.



d, horse serum, etc.

equal degree for sheep and beef blood, and, though in much less degree, for blood of the other species tested.

That the previous stimulation of precipitin-production by sheep blood is largely responsible for the development of so many precipitins after the injection of horse blood, as illustrated in the foregoing, is indicated by the rather strict specificness of the precipitin which develops after the injection of foreign blood in previously untreated animals. Thus in the case of a series of 6 fresh rabbits injected intraperitoneally with 30 c.c. of horse serum, there developed in 4 precipitin waves, which at the height gave titers of 6400-12800 with horse blood and no precipitates at all in dilutions above 1 : 50 with the blood of any of the other species discussed in this article; and, as is well known, results like this are usually obtained from the injection of untreated rabbits with the serum of blood of other species. I have observed, however, that in the fresh rabbit the injection of sheep blood sometimes may result in the production of considerable precipitin for beef blood as well as for sheep blood.

2.—A rabbit, weighing 1050 gm., received 30 c.c. of sheep blood intraperitoneally on May 12, 1915. At the same time it received 1 c.c. of toluene in 1 c.c. of olive oil subcutaneously and this was repeated daily for 12 days. Precipitin for sheep blood developed, the concentration being somewhat less and the fluctuation greater than in the previous case, but the persistence was longer—until somewhere between 122 and 132 days after the injection of the sheep blood.[1] On Day 173 (October 20) 30 c.c. of sheep blood were injected intraperitoneally again and, after a latent period of 3 days, a new wave of precipitin-production set in, reached its high point (12800) about the 12-17th day (Chart 2) after the 2nd injection, and maintained a high level for about 30 days, when 20 c.c. of human blood were injected intraperitoneally, after which it declined rather abruptly. In the early part of January, 1916, about 70 days after the 2nd injection of sheep blood and 243 days after the 1st injection, the precipitin titer was 800 with sheep blood. Practically no precipitin for human blood developed at this time. On Day 250 30 c.c. of horse serum were injected intraperitoneally and abundant precipitin for horse blood developed, the titers on the 10th, 11th, and 12th days being 25-50000 +. Thirteen days later the injection of horse serum was repeated and there resulted a marked drop in the titer, but in a few days however, the titer was again 50000 + for horse blood, and tests with blood of other species now gave the following results: sheep, 3200; beef, 12800; swine, 2560; man, 10000; rat, 1600; dog, 400; guinea-pig, 200; chicken, 160.

In this case an especially noteworthy feature appears to me to be the stimulation by the injection of horse serum of the elaboration of precipitin for human blood, the injection of 20 c.c. of which some weeks previously had been without hardly any recognizable precipitinogenic effect. In this animal as well as in others the titer of the anti-beef precipitin runs especially high.

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[1] This rabbit is the same as Rabbit 5, Table 2, in the article, "The Effect of Toluene on the Production of Antibodies," Jour. Infect. Dis., 1916, 19, p. 737.

3.—The rabbit now considered was 1 of a group which received 10 or more daily injections of toluene 1 c.c. per kilogram of weight before the injection of 30 c.c. of sheep blood intraperitoneally.[2] It developed not even a trace of precipitin, and 171 days after this injection it was given the same amount of sheep blood whereon much precipitin accumulated in the blood in spite of the fact that on the 7th, 8th, 9th, and 10th days after the 2nd injection it was given 1.5 c.c. per kilogram of benzene subcutaneously, and on the 11th and 12th days, 2 c.c. (Chart 3). On the 47th day after the 2nd injection of sheep blood it was given 30 c.c. of horse serum intraperitoneally; on the 96th day, 10 c.c. of guinea-pig blood; on the 105th day, 30 c.c. of horse serum again; and finally on the 136th day, 30 c.c. of sheep blood. Chart 3 gives the results of the observations on precipitins and antisheep lysin.

We note that the beef precipitin, which with the precipitins that are not charted was not estimated until 40 odd days after the second injection of sheep blood, follows quite closely the general course of the sheep and horse precipitins as influenced by injections of horse serum and sheep blood. As set forth by the figures on Chart 3, the injection of guinea-pig serum was succeeded by a wave of specific precipitin which subsided on the injection of sheep blood only to reappear in about 10 days; the other precipitins followed a like course, but the antisheep lysin runs quite independently and seems not to have been influenced by any other antigen than sheep blood.

4.—A young rabbit, weighing about 1000 gm., was injected with 30 c.c. of sheep blood intraperitoneally and 1 c.c. toluene in olive oil subcutaneously. The toluene injection was repeated daily for 12 days in order to study its effect on antibody-production. The subsequent injections and the results of the observations on the antibody-content of the serum are shown in Chart 4. The rabbit increased in weight until the 243rd day when it weighed 2025 gm.; thereafter there was more or less fluctuation and decline. The injections of benzene on Days 96-109 had no effect on the leukocytes or, as far as apparent, on the lysin and precipitin, the remarkedly prolonged persistence of which with great fluctuation as to concentration in the serum may be ascribed at least in part to the effects of the injections of toluene.[3] On the 267th day 30 c.c. sheep blood were injected again and there resulted a prompt and marked increase in antisheep precipitin but not in lysin, which reached normal level while the precipitin was still present in large quantities, its content in the serum being influenced greatly by the subsequent injections of human blood, guinea-pig serum, horse serum, and sheep blood. As a rule there was a fall after each injection succeeded by a rise in all the precipitins considered. No explanation can be advanced for the fall about the 350th day. On the 379th day the left hind leg was removed in order to study the marrow; at this time the

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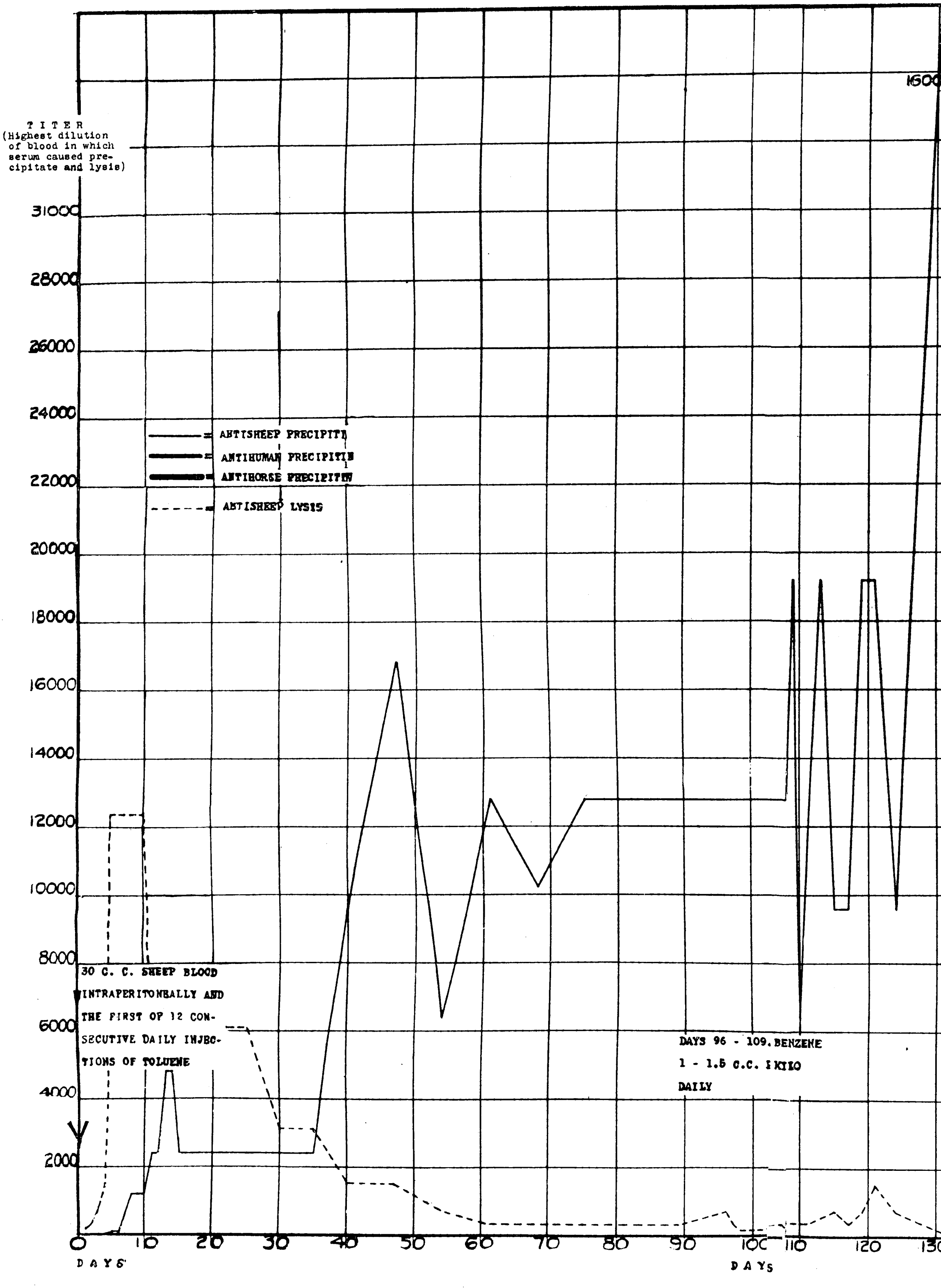
[2] Rabbit 1, Table 3, in the article on toluene (1).

[3] This animal is Rabbit 2, Table 2, in the article on toluene (1).



30 C. C. SHEEP BLOOD INTRAPERITONEALLY AND THE FIRST OF 12 CONSECUTIVE DAILY INJECTIONS OF TOLUENE

DAYS 96 - 109. BENZENE 1 - 1.5 C.C. I KILO DAILY

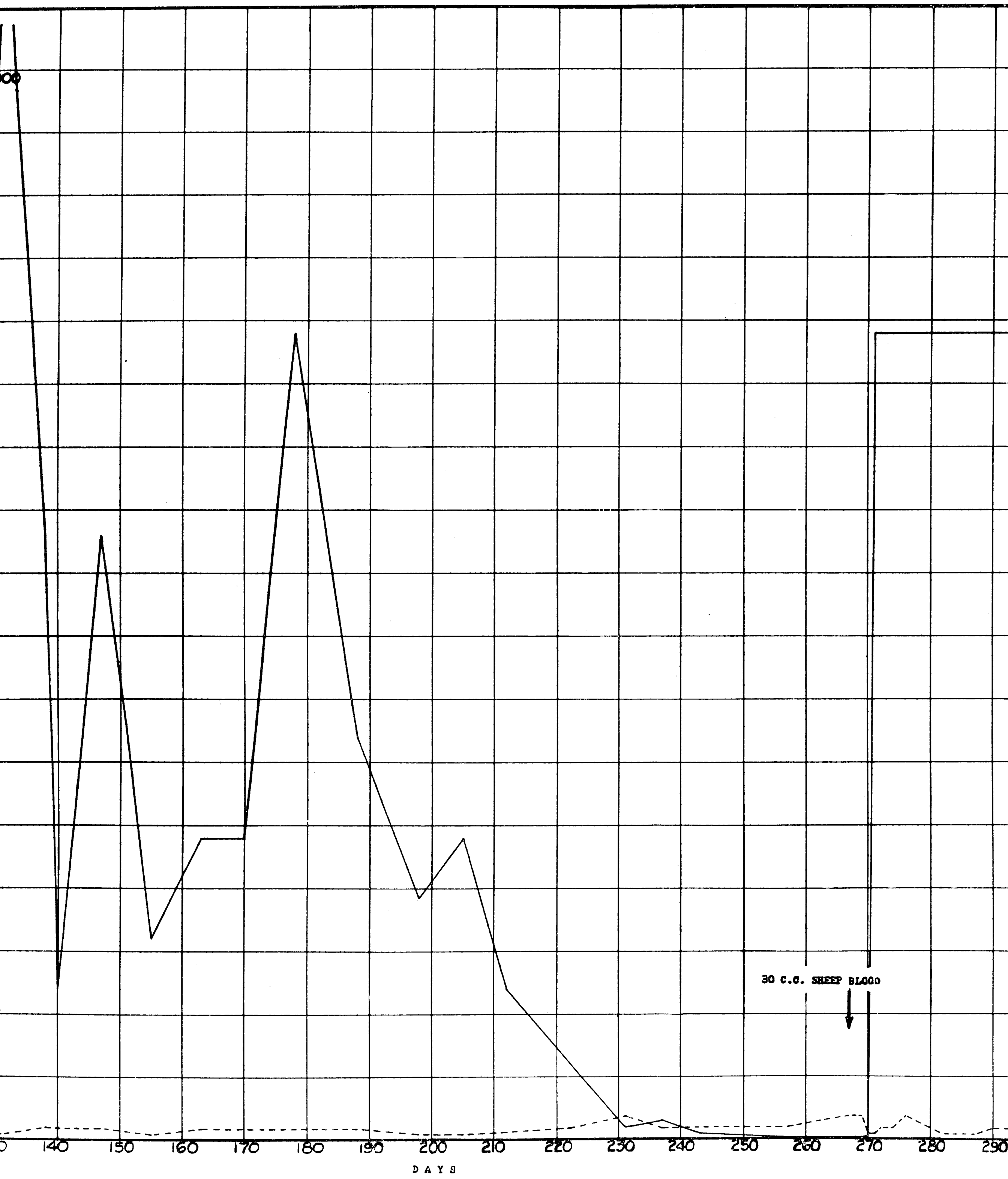
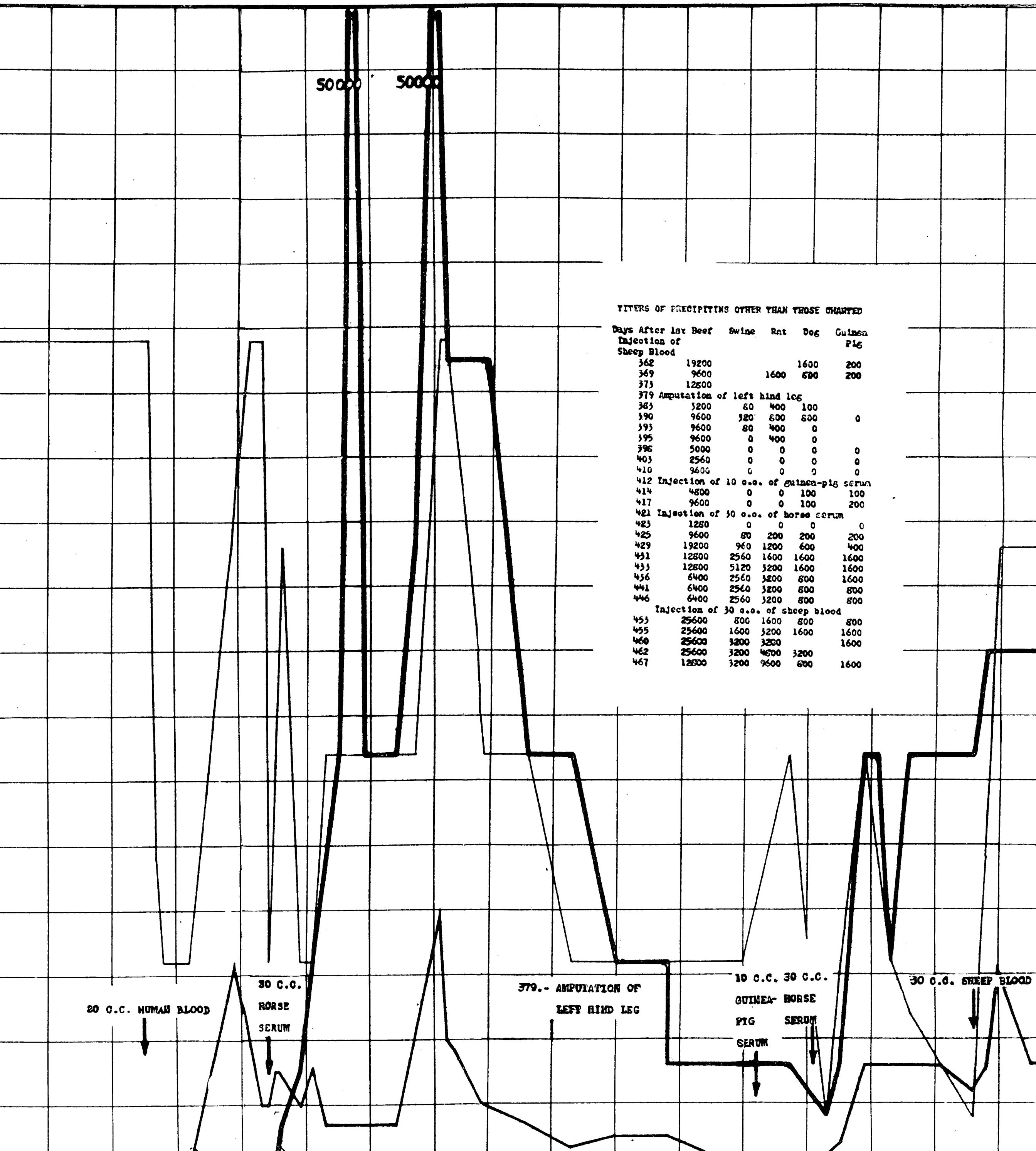
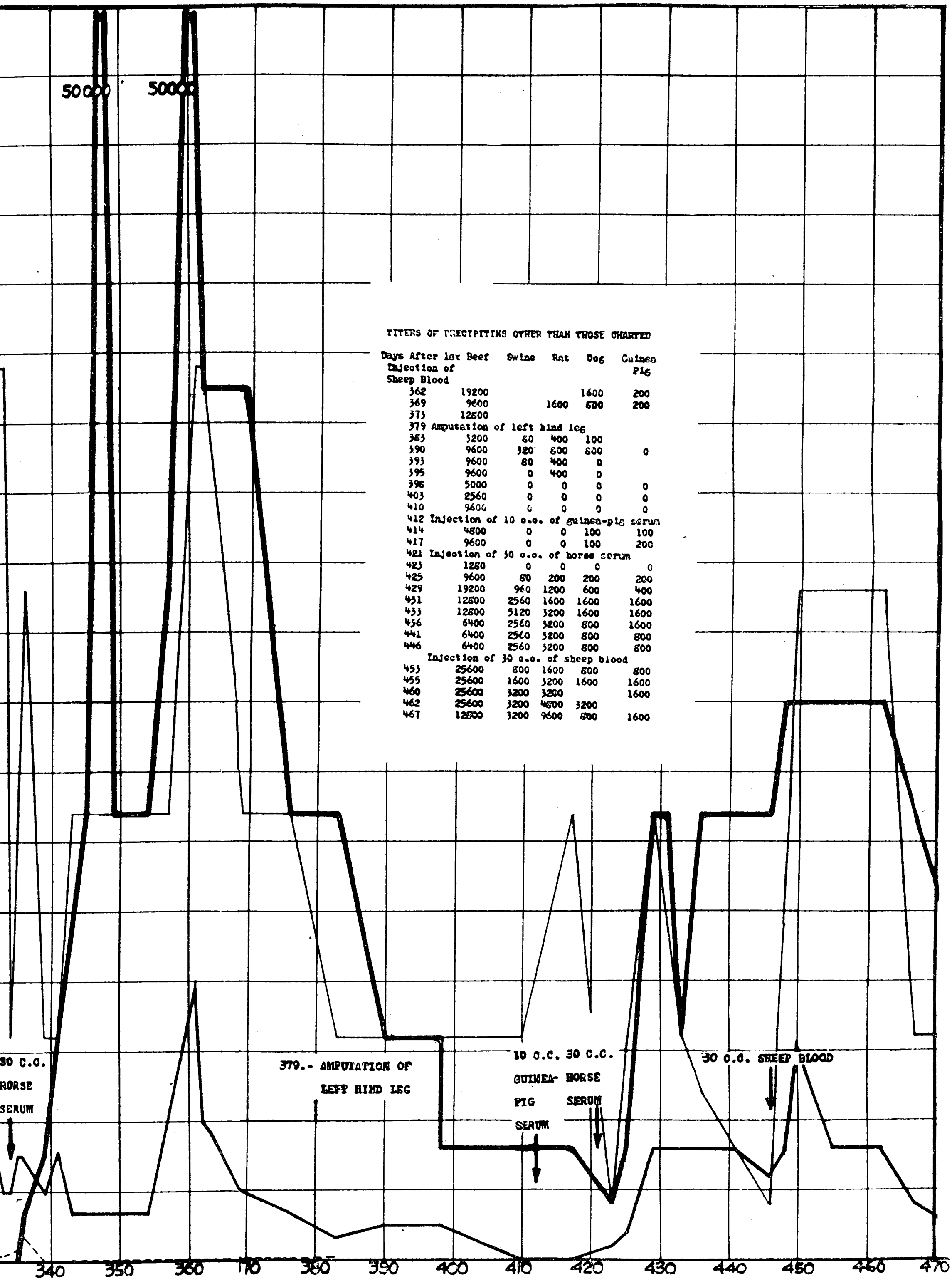


Chart 4. Lysin and precipitins in rabbit injected with sheep blood, human blood, horse serum, etc.



TITERS OF PRECIPITINS OTHER THAN THOSE CHARTED

Days After 1st Injection of Sheep Blood	Beef	Swine	Rat	Dog	Guinea Pig
362	19200			1600	200
369	9600		1600	800	200
373	12800				
379 Amputation of left hind leg					
383	3200	80	400	100	
390	9600	320	800	800	0
393	9600	80	400	0	
395	9600	0	400	0	
396	5000	0	0	0	0
403	2560	0	0	0	0
410	9600	0	0	0	0
412 Injection of 10 c.c. of guinea-pig serum					
414	4800	0	0	100	100
417	9600	0	0	100	200
421 Injection of 30 c.c. of horse serum					
423	1280	0	0	0	0
425	9600	80	200	200	200
429	19200	960	1200	600	400
431	12800	2560	1600	1600	1600
433	12800	5120	3200	1600	1600
456	6400	2560	3200	800	1600
441	6400	2560	3200	800	800
446	6400	2560	3200	800	800
Injection of 30 c.c. of sheep blood					
453	25600	800	1600	800	800
455	25600	1600	3200	1600	1600
460	25600	3200	3200		1600
462	25600	3200	4800	3200	
467	12800	3200	9600	800	1600



TITERS OF PRECIPITINS OTHER THAN THOSE CHARTED

Days After 1st Injection of Sheep Blood	Beef	Swine	Rat	Dog	Guinea Pig
362	19200			1600	200
369	9600		1600	800	200
373	12800				
379	Amputation of left hind leg				
383	3200	80	400	100	
390	9600	320	800	800	0
393	9600	80	400	0	
395	9600	0	400	0	
396	5000	0	0	0	0
403	2560	0	0	0	0
410	9600	0	0	0	0
412	Injection of 10 c.c. of guinea-pig serum				
414	4800	0	0	100	100
417	9600	0	0	100	200
421	Injection of 30 c.c. of horse serum				
423	1280	0	0	0	0
425	9600	80	200	200	200
429	19200	960	1200	600	400
431	12800	2560	1600	1600	1600
433	12800	5120	3200	1600	1600
436	6400	2560	3200	800	1600
441	6400	2560	3200	800	800
446	6400	2560	3200	800	800
	Injection of 30 c.c. of sheep blood				
453	25600	800	1600	800	800
455	25600	1600	3200	1600	1600
460	25600	3200	3200		1600
462	25600	3200	4800	3200	
467	12800	3200	9600	800	1600

blood gave 14,600 leukocytes, 47.5% being granular and 52.5% nongranular. The marrow of the amputated bones seemed about normal except for a rather large number of erythroblasts; there was no increase in eosinophils, giant cells, or kinetic figures.

The extreme divergence in the course of the antisheep lysin and antisheep precipitin in this case points to their being independent substances produced by distinct mechanisms. Johnson[4] records an observation of perhaps similar import in the case of rabbits injected with beef serum, and Gay[5] finds that the lysin in a serum containing precipitin is not brought down on the formation of the specific precipitate, thus showing quite conclusively that the substances in question are distinct.

It is not necessary to try to analyze more minutely than already done the fluctuations in the precipitin and lysin content of the serum of the rabbits under study. The particular point on which special stress is placed now is the capacity of the rabbit under suitable conditions, as shown plainly enough by the charts, to elaborate different precipitins at the same time. It appears that the precipitin-production induced in the usual way leaves behind it an increased power of further production so that large amounts of major as well as group and minor precipitins are elaborated on the injection of a new serum or blood. Manifestly the phenomenon is an expression of an increased reactivity and may be classed with other manifestations of allergy.

Whether the treatment of some of the rabbits with toluene was of much significance in respect to the degree of increased reactivity probably cannot be determined, although such may possibly have been the case; the first observation, however, shows that rabbits not treated with toluene also may develop much increase in the power to elaborate precipitins as the consequence of having passed through a previous course of precipitin-production. I find that in dogs not subjected to the action of toluene, but previously injected intravenously with a single dose of goat blood, the injection of rat blood after the new goat lysin has disappeared may cause a second wave of goat lysin at the same time as the formation of agglutinin for rat corpuscles runs its typical course. Furthermore, in dogs treated with rat blood, injection of goat blood may reawaken the production of rat agglutinin. These results are not obtainable regularly; in an experiment involving 8 dogs, 2 gave the results indicated in Table 1. It should be noted that in this case an altered reactivity is manifested by a resumption of the output of lysin and agglutinin, hence the change is not limited to the renewed production of any particular antibody. This is shown further by the experiments of Conradi and Bieling[6] on rabbits injected with typhoid bacilli; here subsequent injections with colon, dysentery, or diphtheria bacilli resulted in an increase in typhoid agglutinins, and in rabbits injected with paratyphoid bacilli, injection of typhoid bacilli some months later caused rapid increase in paratyphoid agglutinin. We may conclude then that the kind of increased reactivity here discussed is not limited to any particular species or antibody, and that toluene is not essential for its establishment.

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[4] Jour. Immunol., 1916, 1, p. 397.

[5] Ibid., p. 83.

TABLE 1

REACTIVATION OF LYSIN- AND AGGLUTININ-PRODUCTION IN THE DOG BY INJECTION OF BLOOD OTHER THAN THAT FIRST INJECTED *

Days After Second Injection	First Injection, 1 C.c. 10% Suspension of Goat Blood per Kilogram; Second Injection, 1 C.c. 10% Suspension of Rat Blood per Kilogram		First Injection, 1 C.c. 10% Suspension of Rat Blood per Kilogram; Second Injection, 1 C.c. 10% Suspension of Goat Blood per Kilogram	
	Titer of Lysin for Goat Corpuscles	Titer of Agglutinin for Rat Corpuscles	Titer of Agglutinin for Rat Corpuscles	Titer of Lysin for Goat Corpuscles
2	96	96	96	96
4	384	384	1536	384
6	1536	1536	6144	384
8	1536	1536	6144	384
10	1536	1536	6144	384
13	1536	384	3072	384
17	768	192	1536	192
21	192	192	1536	192
31	48	48	768	96
41	48	48	768	96

* The titer gives the highest dilution in which the serum produced lysis and agglutination. Concerning details of tests see Hektoen and Carlson, Jour. Infect. Dis., 1910, 7, p. 319.

These observations are, of course, not without clinical and practical interest. Most recently Conradi and Bieling,[6] in a study of the diagnostic value of the agglutinin test in patients inoculated against typhoid fever, cite numerous examples of marked increase in typhoid agglutinins in patients so inoculated at some previous time, but suffering from nontyphoid infections, for example, miliary tuberculosis, malaria, pneumonia, and erysipelas. It appears as if almost any infection may cause increase in typhoid agglutinin in the inoculated (and in those who have had typhoid fever) — the new infection calls forth agglutinins like those of the past, and under these circumstances a positive agglutination test may not signify typhoid fever at all. Undoubtedly, analogous conditions may arise in human and animal disease with respect to other bacteria than the typhoid bacillus[7] and to proteins other than those of bacterial origin, but at this time it was not the intention to carry the discussion further than to make clear the general bearing of the results from the rabbit experiments.

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[6] Deutsch. med. Wchnschr., 1916, 42, p. 1280.

As stated in the beginning, the serum of rabbits subject to injection of more than 1 kind of serum or blood may come to contain many precipitins in a concentration that far exceeds the limits of quantitative specificity necessary for practical tests as usually conducted. It would seem that as the blood and serum used as antigens in the rabbit experiments contain many different antigenic substances, some of which perhaps are present though in small quantities in all the species concerned, the biologic specificity of the precipitin reaction may become less pronounced, less sharply limited quantitatively, the higher the increased reactivity of the rabbit yielding the precipitin-serum. On this account the serum for practical precipitin tests should be obtained by specific immunization of suitable previously unused rabbits. On the other hand, under certain circumstances it may be found desirable in order to produce antibodies that are not easily put forth, to use allergic animals.

While the injection of foreign blood or serum into a rabbit previously injected with some other blood or serum, may call forth various precipitins in large amounts because of the many antigens in the materials which are injected into an allergic organism, it is also possible that in the allergic animal the course of immunization may have left the mechanisms of antibody-production in such state that they can be aroused again into the same activities as before by new but totally different antigenic stimuli, and possibly even by stimuli of nonantigenic nature. The experiments so far, however, in which efforts have been made to reakawen precipitin-production by means of antigens, regarded as different from that already injected, and by means of metallic colloidal suspensions have given negative results.

SUMMARY

In rabbits previously injected with foreign blood or serum the subsequent injection of a different blood or serum may reawaken the production of precipitins for the antigens first injected, and the serum of such rabbits may be unsuitable for practical precipitin tests because of the wide range of its action.

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[7] In connection with this question see Dreyer and Inman: The Agglutination Curve and Its Importance in the Diagnosis of Typhoid and Paratyphoid Fevers in Inoculated Persons, Lancet, 1917, 192, p. 365. Baehr: Agglutination in Typhus Fever, Jour. Infect. Dis., 1917, 21, p. 21.

Similar manifestations of an increased reactivity may be obtained in dogs as regards lysin and agglutinin for goat and rat corpuscles, respectively, as well as in rabbits and human beings with respect to typhoid agglutinins, and the increase of typhoid agglutinins in infectious conditions in persons previously inoculated or infected with typhoid bacilli may not signify typhoid fever.